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Final Report: Breaking the Neural Code

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5e. TASK NUMBER

5f. WORK UNIT NUMBER

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The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
This seedling proposed to use advanced imaging techniques to break the neuronal code that links the firing of neurons in the cerebral cortex with behavior. We proposed to use holographic two-photon excitation and microscopy in awake behaving mice to directly modify and create activity patterns in neurons of the primary visual cortex to alter perception and decision making. Our work combined sophisticated optical imaging and photostimulation technologies with state-of-the-art computational detection of the activity with prediction and computation of the precise activity patterns that proved to be most effective at altering a simple visual behavioral

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Spatial Light Moulators (SLM), Open Loop Stimulation, Closed Loop Stimutation, Optogeneics

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Rafael Yuste
UU	UU	UU	UU		19b. TELEPHONE NUMBER 212-854-2354

Report Title

Final Report: Breaking the Neural Code

ABSTRACT

This seedling proposed to use advanced imaging techniques to break the neuronal code that links the firing of neurons in the cerebral cortex with behavior. We proposed to use holographic two-photon excitation and microscopy in awake behaving mice to directly modify and create activity patterns in neurons of the primary visual cortex to alter perception and decision making. Our work combined sophisticated optical imaging and photostimulation technologies with state-of-the-art computational detection of the activity with prediction and computation of the precise activity patterns that proved to be most effective at altering a simple visual behavioral choice. The Yuste and Paninski laboratories combined their efforts in this interdisciplinary project, with the ultimate goal of decoding and modulating the neuronal activity patterns by generating a closed-loop on-line experimental platform. We have completed all proposed tasks of the seedling and successfully completed preliminary closed-loop stimulation. Recent results are included in this final report. We have published 2 journal articles and have three manuscripts in submission form the funded work.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
05/15/2015	1.00	J.-e. K. Miller, I. Ayzenshtat, L. Carrillo-Reid, R. Yuste. Visual stimuli recruit intrinsically generated cortical ensembles, Proceedings of the National Academy of Sciences, (09 2014): 4054. doi: 10.1073/pnas.1406077111
05/18/2015	4.00	Luis Carrillo-Reid, Jae-eun Kang Miller, Jordan P. Hamm, Jesse Jackson, Rafael Yuste. Endogenous Sequential Cortical Patterns Evoked by VisualStimuli, Journal of Neuroscience, (05 2015): 0. doi:
TOTAL:		**2**

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received		Paper
TOTAL:		

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received		Paper
05/15/2015	2.00	Weijian Yang, Jae-eun Miller, Luis Carillo-Reid, Eftychios Pnevmatikakis, Liam Paninski, Rafael Yuste, Darcy S. Peterka. Simultaneous multi-plane imaging of neural circuits, Neuron (05 2015)
05/15/2015	3.00	Eftychios A. Pnevmatikakis, Daniel Soudry, Yuanjun Gao, Tim Machado, David Pfau, Thomas Reardon, Yu Mu, Clay Lacefield, Kira E. Poskanzer, Misha Ahrens, Darcy S. Peterka, Randy Bruno, Tom Jessell, Rafael Yuste, Liam Paninski. Simultaneous denoising, deconvolution, and demixing of calcium imaging data, Neuron (05 2015)
TOTAL:	**2**	

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Rafael Yuste:
Spanish Royal Academy of Sciences Member

Jimemez Diaz award

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Ruoxi Sun	0.40	
FTE Equivalent:	**0.40**	
Total Number:	**1**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Luis Carillo Reid	1.00
Ekaterina Taralova	0.50
Alexander Dubbs	0.50
Evan Archer	0.50
Ari Pakman	0.30
Eftychios Pnevmatikakis	0.20
FTE Equivalent:	**3.00**
Total Number:	**6**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Liam Paninski	0.16	
FTE Equivalent:	**0.16**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
William Snider	0.10	Biological Sciences
FTE Equivalent:	**0.10**	
Total Number:	**1**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: 0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
Reka Racinos	0.20
FTE Equivalent:	**0.20**
Total Number:	**1**

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

Miller et. al. (2015)

Significance:
This study demonstrates that neuronal groups or ensembles, rather than individual neurons, are emergent functional units of cortical activity. We show that in the presence and absence of visual stimulation, cortical activity is dominated by coactive groups of neurons forming ensembles. These ensembles are flexible and cannot be accounted for by the independent firing properties of neurons in isolation. Intrinsically generated ensembles and stimulus-evoked ensembles are similar, with one main difference: Whereas intrinsic ensembles recur at random time intervals, visually evoked ensembles are time-locked to stimuli. We propose that visual stimuli recruit endogenously generated ensembles to represent visual attributes.

Major Findings:
We performed two-photon calcium imaging of populations of neurons from the primary visual cortex of awake mice during visual stimulation and spontaneous activity. Under both conditions, cortical activity is dominated by coactive groups of neurons, forming ensembles whose activation cannot be explained by the independent firing properties of their contributing neurons, considered in isolation.

Individual Neurons Contribute Flexibly to Multiple Ensembles.
Individual neurons flexibly join multiple ensembles, vastly expanding the encoding potential of the circuit.

Visually Evoked Ensembles Are Similar to Spontaneous Ensembles
The same coactive ensembles can repeat spontaneously and in response to visual stimuli, indicating that stimulus-evoked responses arise from activating these intrinsic building blocks.
Although the spatial properties of stimulus-driven and spontaneous ensembles are similar, spontaneous ensembles are active at random intervals, whereas visually evoked ensembles are time-locked to stimuli.

Conclusion:
Neuronal ensembles, built by the co-activation of flexible groups of neurons, are emergent functional units of cortical activity and propose that visual stimuli recruit intrinsically generated ensembles to represent visual attributes

Yang et. al. (2015)

Significance:
We are optimistic about the future of volumetric imaging in scattering tissue, and consider SLM-based multiregion imaging as but one implementation of a general strategy of computationally enhanced projective imaging, which will make possible the ability to interrogate neurons over a very large area, with high temporal resolution and SNR.

Major Findings:
Imaging the functional activity of large populations of neurons within the brain could be critical towards understanding the structure and function of neural circuits.

Here we present a simple holographic microscopy method to simultaneously image the neuronal activity of the mouse cortex in vivo, across multiple cortical layers (L2/3 and L5).

We demonstrate successful simultaneous 3D multilayer in vivo imaging with a hybrid SLM multibeam-scanning approach that leverages spatiotemporal sparseness of activity and prior structural information to efficiently extract single cell neuronal activity.

We can either extend the effective area that can be sampled, target multiple axial planes over an extended range, > 500 ⌣m, or both, at depth within the cortex.

The regional targeting is performed remotely, through holography, without any motion of the objective, which makes the technique a strong complement to 3-D two-photon activation.

The module allows for simple and rapid software based multiplexing, with low cost, and offers considerable flexibility and performance for a variety of imaging paradigms, and creates a powerful tool to study the functional activity of neural circuits across multiple areas in the brain.

Conclusion:
This imaging approach – multiplexed excitation combined with computational source separation and reconstruction – may be ideal for future high speed in vivo volumetric imaging in scattering tissue.

Carillo Reid et. al. (2015)

Major Findings:
Although the functional properties of individual neurons in primary visual cortex have been studied intensely, little is known about how neuronal groups could encode changing visual stimuli using temporal activity patterns.

Identification of neuronal ensembles in multineuronal recordings
We used in vivo two-photon calcium imaging to record the activity of neuronal populations in primary visual cortex of awake mice in the presence and absence of visual stimulation. Multidimensional analysis of the network activity allowed us to identify neuronal ensembles defined as groups of cells firing in synchrony.
Evoked neuronal ensembles recapitulate spontaneous ones
These synchronous groups of neurons were themselves activated in sequential temporal patterns, which repeated at much higher proportions than chance and were triggered by specific visual stimuli such as natural visual scenes.
Hebbian cell assemblies in cortical microcircuits
Sequential patterns were also present in recordings of spontaneous activity without any sensory stimulation and were accompanied by precise firing sequences at the single-cell level. Intrinsic dynamics could be used to predict the occurrence of future neuronal ensembles.

Conclusion:
Our data demonstrate that visual stimuli recruit similar sequential patterns to the ones observed spontaneously, consistent with the Hebbian hypothesis that already existing neuronal ensembles firing in predefined temporal sequences could be the microcircuit substrate that encodes visual percepts changing in time.

Pnevmatikakis et. al. (2015)

Significance:
Calcium imaging as a neural recording method can provide rich and diverse datasets depending on many factors (imaging technique, experimental conditions, calcium indicators etc), and multineuronal ground truth spiking data is scarce and typically hard to obtain. As such, the goal of a globally operating algorithm that requires no human intervention and has performance guarantees remains elusive. Nevertheless, we hope that the family of methods discussed here, under the unifying umbrella of constrained matrix factorization, will provide a useful framework for future research.

Major Findings:
Developed a modular approach for analyzing calcium imaging recordings of large neuronal ensembles.

A flexible and efficient model for calcium deconvolution
Simultaneous identification of the locations of the neurons, demix spatially overlapping components, and denoise and deconvolve the spiking activity from the slow dynamics of the calcium indicator. This builds upon and extends the fast non-negative deconvolution (FOOPSI) method of Vogelstein.

Constrained matrix factorization provides a flexible framework for analysis of large-scale calcium imaging data
We developed a matrix factorization approach that expresses the spatiotemporal fluorescence activity as the product of a spatial matrix that encodes the spatial footprint of each neuron in the optical field and a temporal matrix that characterizes the calcium concentration of each neuron over time.

Segmentation of large-scale video data
Using in vivo mouse V1 spontaneous activity data and the greedy algorithm which efficiently identifies neurons with very few visually-apparent false positives and denoises the calcium activity of each cell.

A modular approach towards automated calcium imaging data analysis
This framework is combined with a novel constrained deconvolution approach that extracts estimates of neural activity from fluorescence traces, to create a spatiotemporal processing algorithm that requires minimal parameter tuning. A specific objective in this work was to create a framework that requires minimal human intervention

Conclusion:
We demonstrate the general applicability of our method by applying it to in vitro and in vivo multi-neuronal imaging data, whole brain light-sheet imaging data, and dendritic imaging data.

Technology Transfer

Scientific progress and accomplishments

(1) Foreword (optional)
(2) Table of Contents (NA)
(3) List of Appendixes, Illustrations and Tables (if applicable)

Figure 1- Mulitplane SLM microscope schematic and data (Yang et. al. 2015 submitted manuscript Neuron)

Figure 2- NMF Method with mouse V1 data

(4) Statement of the problem(s) studied:

(Yuste) How do neuronal groups encode changing visual stimuli using temporal activity patterns? What new technologies can we develop so we can see wider and deeper in mouse brains?

(Paninksi) Calcium imaging pose significant challenges to statistical neuroscientists, there are 3 major problems: (i) identifying the spatial footprint of each neuron in the optical field, (ii) demixing spatially overlapping neurons (where overlap is due either to the projection of a 3d volume onto a 2d imaging plane, or to insufficient spatial resolution in 3d imaging methods) and (iii) deconvolving the spiking activity of each neuron from the much slower dynamics of the calcium indicator.

(5) Summary of the most important results – highlights of the papers and manuscripts.

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(6) Bibliography
NA

(7) Appendixes

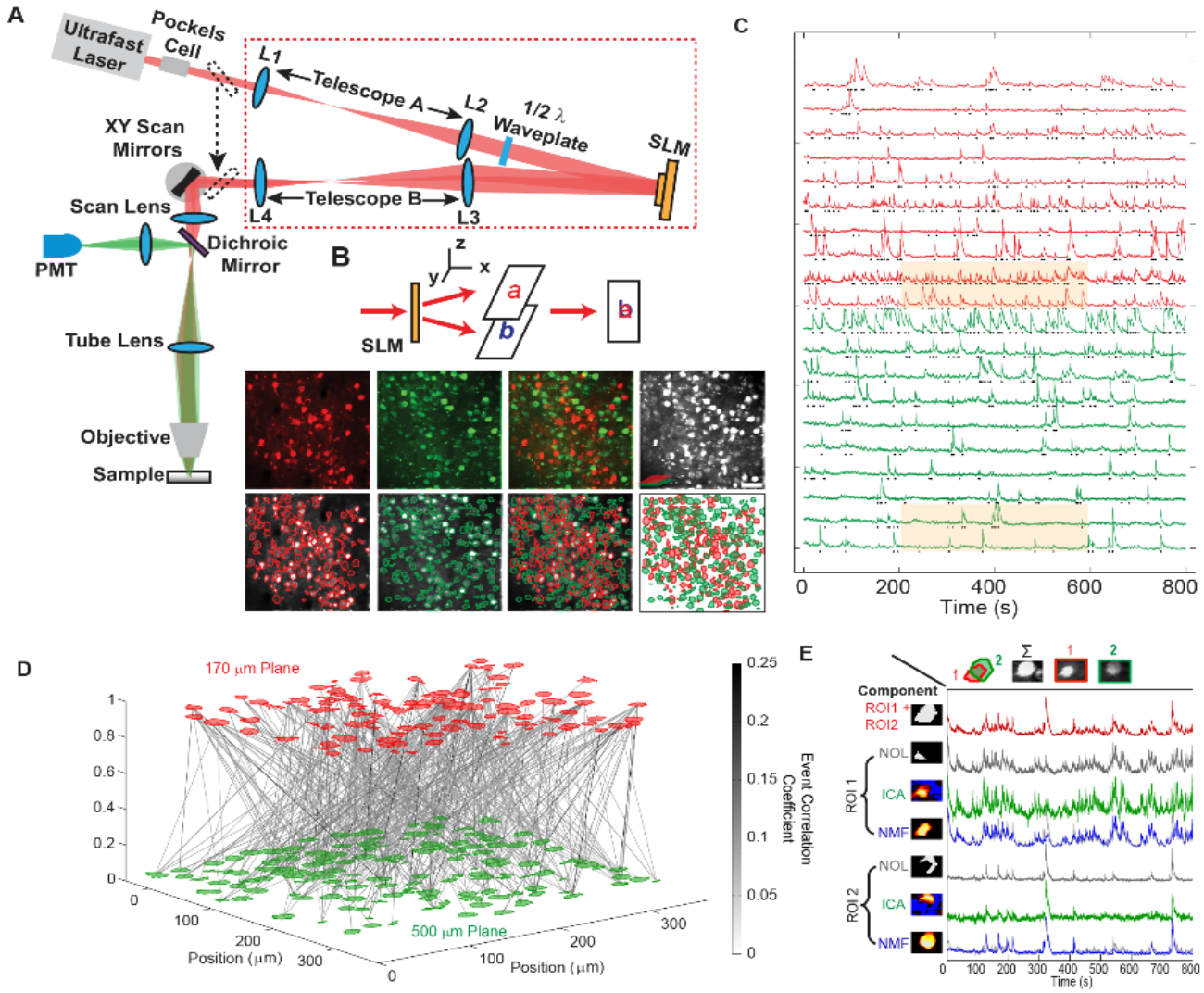

Figure 1: A) Schematic of multi-plane microscope. B) Cartoon, and actual data set of simultaneous dual plane imaging, here L2/3 and L5 of mouse visual cortex, taken at 10 Hz. C) Extracted activity traces, color coded for each plane. Note extremely high SNR D) Interlaminar correlation map between L2/3 and L5 neurons. E) Demonstration of NMF source separation vs human chosen non-overlapped ROIs and ICA extracted signals. NMF performs better than both. (Adapted from Yang et. al. 2015 submitted *Neuron*)

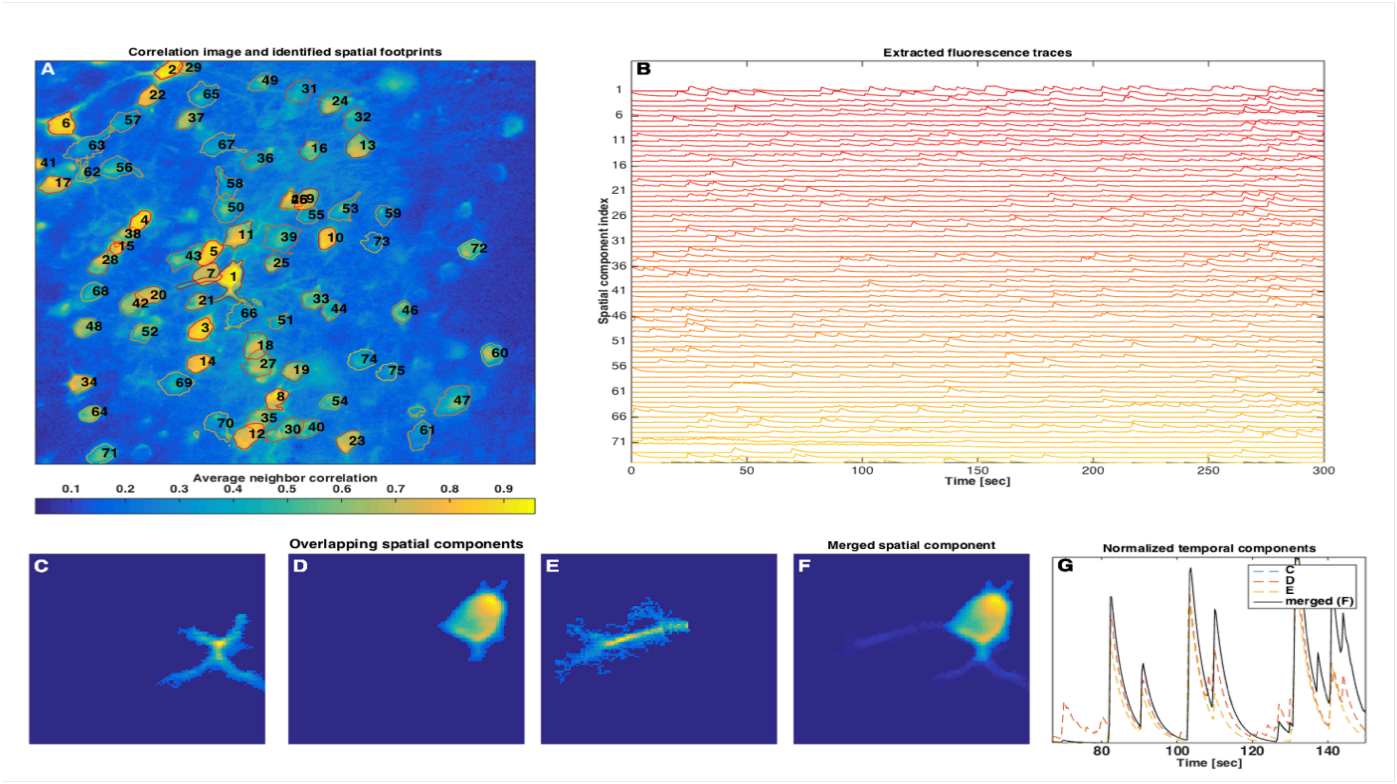

Figure 2. Application to mouse V1 in vivo data. A: Inferred spatial footprints superimposed on the correlation image of the raw data. The components are sorted in decreasing order based on the maximum temporal value. B: Extracted (normalized) fluorescence traces from the corresponding spatial components. C-G: Depiction of the merging operation: C-E: Three overlapping spatial components with highly correlated temporal components. F: Merged spatial component (component 1 is panels A-B). G: Estimated temporal components of individual (dashed) and merged (solid) components. The constrained NMF method can efficiently identify multiple neurons with possibly overlapping spatial footprints. See Supplementary video S1 for a clearer spatiotemporal view of these results. (From Pnevmatikakis et. al. 2015 submitted *Neuron*)